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First synthesis of etidronate partial amides starting from PCl_3

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Methods for the preparation of mixed tetra-amide esters **1 and 2**, the partial amide ester **3**, and tri- and *P*,*P*-diamides **4 and 5** from monophosphorus spieces **12**, **8** and **9**, respectively, were developed. Compounds **8** and **9** were obtained from phosphorus trichloride *via* $MeOPCl_2$, which was treated with 2 eq. and 4 eq. of piperidine, followed by water or acetyl chloride, respectively. Tetrasubstituted amide bisphosphonates **1 and 2** were selectively dealkylated with lithium or silyl halide to achieve target compounds **3**–**5**. Piperidine was found to be a good desilylation reagent. Quantum mechanical calculations illustrate why derivative **2** was produced in low yield. The usefulness of compounds **1**, **3** and **4** as prodrugs of etidronate was determined in aqueous buffer and human serum.

Introduction

Chemically and enzymatically stable bisphosphonates, characterized by a P–C–P bridge, are analogues of pyrophosphate.[1] These compounds bind strongly to calcium phosphate and inhibit its formation, aggregation and dissolution. Their affinity to bone mineral is the basis for their use as skeletal markers and inhibitors of bone resorption. Bisphosphonates are the most effective inhibitors of osteoclastic bone resorption, and are therefore useful drugs in both the treatment and prevention of osteoporosis.[3] Etidronate, clodronate, alendronate and pamidronate are examples of these drugs which are used in various bone diseases.[1,3]

Etidronate, (1-hydroxyethylidene)-1,1-bisphosphonic acid (HEBPA) disodium salt, is highly hydrophilic and like other tetraacidic bisphosphonates, its oral bioavailability is poor; only 3–7% of the dose is absorbed.[4] One way to increase the lipophilicity and oral absorption is to prepare more lipophilic compounds, such as the amide derivatives **1**–**5** shown in Fig. 1, which might function as prodrugs of etidronate. A prodrug is a drug derivative that requires a chemical or enzymatic biotransformation to release the parent drug.[5] Recently, pivaloyloxymethyl derivatives of HEBPA were reported as potential prodrugs of HEBPA.[6]

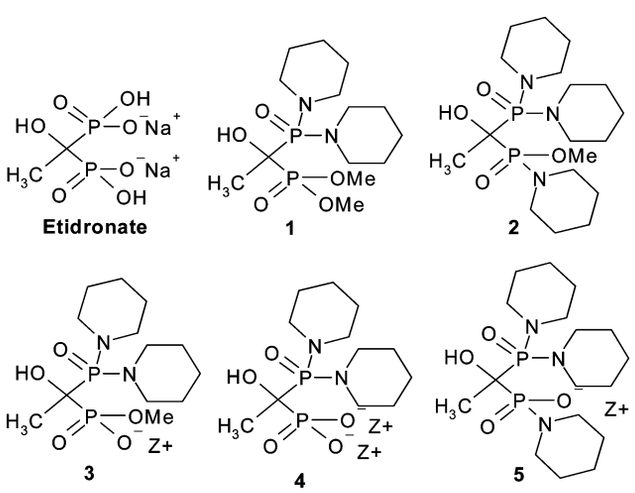


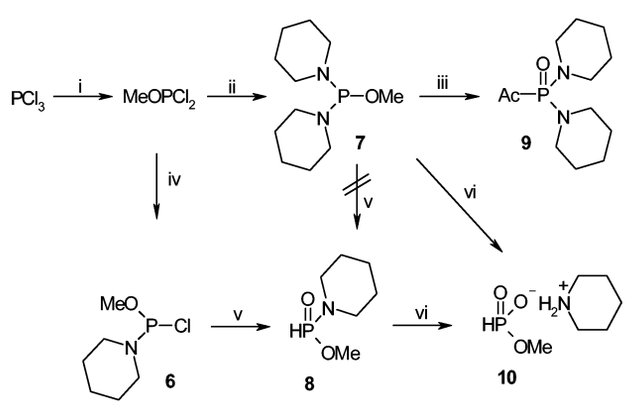
Fig. 1 Prepared etidronate derivatives.

Results and discussion

New amide derivatives of etidronate were prepared from monophosphorus starting materials **8** and **9** according to previously described procedures.[7] However, these two compounds were previously unknown derivatives of H–P(O)– and AcP(O)-structures. Both **8** and **9** were obtained *via* $MeOPCl_2$, which was prepared from PCl_3 and methanol. Preparation of the piperidine derivatives in the next step was more problematic than expected due to rearrangement of P(III) to P(V) and easy cleavage of the P–N bond.[8,9]

The yield and purity of compound **7** was dependent on both the reaction temperature and formation of the P–N bond from the P–Cl, as reaction of the first two clorine atoms of PCl_3 with piperidine and subsequent treatment of the Cl–$P(NR_2)_2$-derivative with MeOH lead to a mixture of compounds, due to an addition–elimination reaction between MeOH and trivalent phosphorus amide.[8] This results in the elimination of the amine from phosphorus amide and addition of MeOH to the trivalent phosphorus. In preparing compound **9** at room temperature or at 0 °C, *N*-acetylpiperidine and compound **6** were observed to be the main products (see Scheme 1).

Rather good results were obtained by adding acetyl chloride to compound **7** in ether at −30 °C giving **9** as the main product over *N*-acetylpiperidine and **6**. Similar problems were met in



Scheme 1 *Reagents and conditions*: i) MeOH; ii) 4 eq. piperidine; iii) acetyl chloride, −30 °C; iv) 2 eq. piperidine; v) 1.05 eq. H_2O in 1.1 eq. triethyl amine, 0 °C; vi) H_2O.

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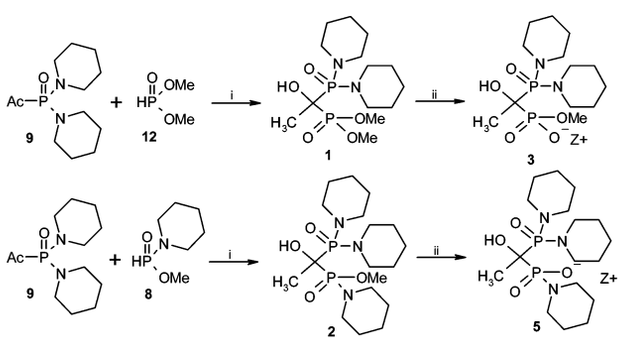
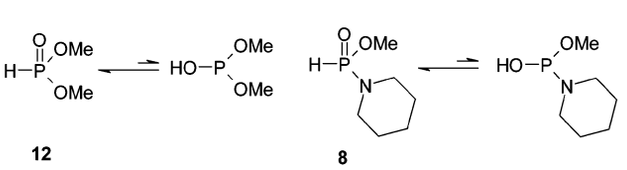
preparing **8** from **7** by adding water, which is a common procedure to obtain H–P(O)-compounds from trivalent phosphites.[7a,10] In the case of **7**, both of the P–N bonds were cleaved, resulting in the methyl phosphite **10**, and only trace amounts of **8** were observed. Starting from **6**, which was prepared from $MeOPCl_2$ and 2 eq. of piperidine, the production of **8** was more successful as the more reactive chlorine atom was replaced by OH and after rearrangement **8** was observed at reasonable yield (see Scheme 1).

The bisphosphonate starting materials **1** and **2** were obtained by a known method[7] from the compounds above with dibutylamine as catalyst (Scheme 2). Compound **1** was obtained in 55% yield while only about 9% of derivative **2** was isolated. Instability or lower reactivity of **8** may be reasons for the low yield of **2**. The lower reactivity of **8** offers possibilities for side-reactions, which were actually observed in the ^{31}P NMR spectrum when preparing compound **2**. This reaction was expected to follow a mechanism reported earlier,[7a,11] and quantum mechanical calculations were made to examine some reasons why derivative **2** was obtained only in 9% yield under these conditions.



Scheme 2 Preparation of the compounds **3** and **5**. *Reagents and conditions*: i) Bu_2NH; ii) LiI–acetone.

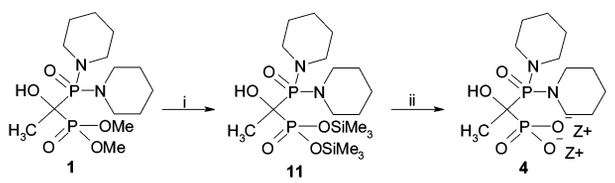
The quantum mechanical calculations were carried out to estimate relative stabilities of the P(V) and P(III) tautomers of **12** and **8** (Scheme 3). The P(III) tautomers are thought to be the reactive species in the mechanism leading to **1** and **2**. As expected, the P(V) tautomers of both **12** and **8** are more stable than their P(III) tautomers. For **12**, the energy difference in the gas phase was 32.6 kJ mol^{-1} at the MP2/6-311G**//HF/6-31G* level and 16.9 kJ mol^{-1} at the B3LYP/6-311G**//B3LYP/6-31G* level. Solvation by diethyl ether was estimated to stabilise the P(III) tautomer of **12** by 2.5 kJ mol^{-1} (at the HF/6-31G* level) compared to P(V). The P(III) tautomer of **8** was calculated to be less stable than that of **12**. In the gas phase the energy difference was 0.8 kJ mol^{-1} (MP2/6-311G**//HF/6-31G*) and 4.7 kJ mol^{-1} (B3LYP/6-311G**//B3LYP/6-31G*). Inclusion of solvation energies increased this difference to 5.3 kJ mol^{-1} and 9.2 kJ mol^{-1}, respectively. On the basis of these calculations, the lower population of reactive P(III) tautomer for **8**, relative to **12**, leads to 1–2 orders of magnitude lower reactivity for **8** in the reaction, which partly explains the lower yield of **2** when compared to **1**. The steric bulk of the N substituent is likely to play a role in the sterically crowded transition state leading to compound **2**.



Scheme 3 Tautomers of the dimethyl phosphite (**12**) and piperidin-1-yl phosphinic acid methyl ester (**8**).

Recently, we have developed a method to selectively prepare partial esters of HEBPA,[12] and the same approach was also successful for synthesizing compound **3**, the first partial amide ester of HEBPA. This derivative was prepared from **1** using lithium iodide as a demethylation agent, affording **3** as the only product (Scheme 2) without any degradation of the phosphonamide bond. Surprisingly, the same procedure gave **5** from **2**, but the yield was rather low due to subsequent purification problems (Scheme 2).

If both of the methyl groups need to be removed, the silylation method[12–14] was found to be useful for dealkylation over piperidine groups, but hydrolysis of silyl groups by MeOH did not lead to **4** as a free acid, since P–N bonds tended to hydrolyse as well. In contrast, when piperidine was used as the desilylating reagent for the compound **11**, the piperidinium salt of **4** was the only product (see Scheme 4 and Experimental).



Scheme 4 *Reagents and conditions*: i) trimethylsilyl bromide, rt; ii) piperidine.

^{31}P NMR spectroscopy was used to follow the formation of HEBPA amides and the purity of the compounds was analysed by ^{1}H and ^{31}P NMR spectra. Some characteristic features were found in the proton spectra; namely the unequal chemical shifts of the P–N–C*H*$_2$–R methylene protons due to prochirality, which gives rise to a complex splitting pattern, as previously reported for tetra-amide esters and partial amides of methylene- and (dichloromethylene)bisphoshonates.[15] The triplet observed at 3.47 ppm for the tetra-amide ester **1** belonging to the O*H*-proton indicates a strong hydrogen bond from the O*H*-proton to the phosphorous double bonded oxygen, as some tetra-esters we have recently published.[7a]

By definition, prodrugs are pharmacologically inactive compounds that are able to release the active parent drug (in this case etidronic acid) *via* a chemical or enzymatic reaction. To evaluate the usefulness of compounds **1**, **3** and **4** as prodrugs of etidronate, we studied their hydrolysis kinetics and ability to release etidronic acid in aqueous buffer and in 80% human serum. Compounds **1** and **3** had half-lives of 4.2 h and 7.3 h in 50 mM phosphate buffer (pH 7.4, 37 °C). However, etidronic acid was not released, nor was it in the hydrolysis experiments in 80% human serum. Hydrolysis in both experiments apparently resulted in the formation of the corresponding mono- or dimethyl esters. These products were chemically and enzymatically stable, which was previously observed with simple esters of clodronic acid.[16] Therefore, compounds **1** and **3** are not prodrugs of etidronate. Compound **4** degraded totally to etidronic acid in 50 mM phosphate buffer (pH 7.4; 37 °C) in 5 minutes, and can be considered as an etidronate prodrug. However, its usefulness is questionable, due to its low chemical stability.

Conclusions

Two methods for the first synthesis of partial amides (**4** and **5**) and a partial amide ester (**3**) of HEBPA have been developed. Lithium iodide and trimethylsilyl bromide were used as demethylation reagents, and piperidine was noticed to be very useful agent for desilylation in these reactions. Quantum mechanical calculations and observed side-reactions in the reaction mixture at least partly explains the low yield of **2** under these reaction conditions.

Experimental

General

Solvents were HPLC-grade and were used without further purification. The dimethyl phosphite (**12**) which was used to prepare compound **1** is commercially available. Tubes filled with anhydrous $CaCl_2$ were used to protect reactions from outside humidity if not stated otherwise. ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.1, 202.5 and 125.8 MHz, respectively. TMS was used as an internal standard for ^{1}H and ^{13}C measurements, and 85% H_3PO_4 was used as an external standard for ^{31}P measurements. The $^3J_{HH}$ couplings are indicated by the letter "*J*" and all *J* values are given in Hz. The number of protons on each carbon were detected from DEPT-135 experiments and marked after each carbon by the letters d (doublet), t (triplet) or q (quartet). The $''J_{CP}$ couplings were calculated from carbon spectra and the coupling constants are given in parenthesis as hertz. The purity of products was determined from ^{1}H and ^{31}P NMR spectra and was ≥95% unless stated otherwise. Elemental analyses were determined for compounds **1** and **3**. Synthesis yields were not optimised. The methods used in prodrug evaluation are described elsewhere.[6]

All the quantum mechanical calculations were done with the Gaussian 98 (Rev. A.7) program.[17] The geometries of the molecules were energy-minimised at the HF/6-31G* and B3LYP/6-31G* levels followed by single-point energy calculations at the MP2/6-311G** and B3LYP/6-311G** levels, respectively. Several conformations of all the molecules were considered and the reported results are calculated from lowest energy conformations. To estimate the effect of solvation on energies, single-point calculations were made for the gas-phase optimised structures using the polarisable continuum model (PCM) of Tomasi and co-workers.[18,19] The dieletric constant for diethyl ether ($\varepsilon = 4.335$) was used in the solvation energy calculations.

Methyl(piperidin-1-yl)phosphinous chloride (6). Prepared from 1 eq. of $MeOPCl_2$ and 2 eq. of piperidine, giving **6** as a colourless liquid (see preparation of **8**). ^{31}P NMR ($CDCl_3$) δ 174.32.

Dipiperidin-1-yl methyl phosphite (7). PCl_3 (16 g, 0.12 mol) was dissolved in ether (100 ml) and cooled to 0 °C under a nitrogen atmosphere. Methanol (4.3 g, 0.13 mol) was slowly introduced with stirring. The reaction mixture was allowed to warm to room temperature with continuous stirring for 2 hours. Ether was first removed by distillation using a 15 cm fraction column, and the residue was further distilled to give $MeOPCl_2$ (8.0 g, 0.060 mol, 50% bp 91–93 °C), which was then dissolved in ether (200 ml), treated with piperidine (21.3 g, 0.25 mol) and stirred for 48 hours at room temperature. The resulting precipitate was filtered, and ether was removed to give **7** (8.4 g, 60%) as a slightly yellow oil at 91% purity. ^{1}H NMR ($CDCl_3$) δ 3.40 (3H, d, $^3J_{HP}$ = 12.7), 2.95 (8H, m), 1.56 (4H, m), 1.45 (8H,m); ^{31}P NMR ($CDCl_3$) δ 131.94; ^{13}C NMR ($CDCl_3$) δ 51.57 qd ($^2J_{CP}$ = 16.6), 45.59 td ($^2J_{CP}$ = 16.8), 27.19 td ($^3J_{CP}$ = 5.1), 25.53 t.

Piperidin-1-yl phosphinic acid methyl ester (8). $MeOPCl_2$ (3.7 g, 0.028 mol) was dissolved in ether (100 ml) and cooled to 0 °C under a nitrogen atmosphere. Piperidine (4.7 g, 0.055 mol) was slowly introduced with rapid stirring. The reaction mixture was allowed to warm to room temperature and stirring was continued for 1.5 hours. Ether (80 ml) was added and the reaction mixture was cooled to 0 °C. Triethylamine (3.1 g, 0.031 mol) in water (0.53 g, 0.029 mol) was slowly introduced to the mixture at this temperature with stirring and the resulting reaction mixture was allowed to warm to room temperature. Stirring was continued for 2.5 hours, then filtered and the filtrate was evaporated *in vacuo*. The residue was stirred with hexane (30 ml) for 15 minutes and then placed into the freezer (−20 °C) for 2 hours. The hexane layer was separated, evaporated in *vacuo* and distilled under reduced pressure to give **8** [1.07 g, 24% (calculated from $MeOPCl_2$), bp 51 °C/4.6^{-1} mbar] as a colourless liquid. ^{1}H NMR ($CDCl_3$) δ 6.72 (1H, d, $^1J_{HP}$ = 638.9), 3.65 (3H, d, $^3J_{HP}$ = 12.1), 3.09 (4H, m), 1.62 (2H, m), 1.53 (4H, m); ^{31}P NMR ($CDCl_3$) δ 15.45; ^{13}C NMR ($CDCl_3$) δ 50.10 qd ($^2J_{CP}$ = 6.2), 43.46 td ($^2J_{CP}$ = 3.7), 26.15 td ($^3J_{CP}$ = 4.2), 24.57 t.

1-(Dipiperidin-1-yl-phosphinyl)ethanone (9). Compound **7** (3.95 g, 0.017 mol) was dissolved in ether (25 ml), and acetyl chloride (1.33 g, 0.017 mol) was added slowly at −30 °C. The reaction mixture was allowed to warm to room temperature and the ether was removed *in vacuo*. Ethyl acetate was added and the resulting precipitate was filtered; this step was repeated three times. *N*-acetylpiperidine was removed *in vacuo* by stirring at 40 °C for 24 hours. The residue was dissolved in hexane and the solution was separated over formed solids and evaporated *in vacuo* to give **9** (2.8 g, 63%) as a orange oil at 92% purity. ^{1}H NMR ($CDCl_3$) δ 3.03 (8H, m), 2.49 (3H, d, $^3J_{HP}$ = 4.2) 1.59 (4H, m), 1.51 (8H, m); ^{31}P NMR ($CDCl_3$) δ 12.17; ^{13}C NMR ($CDCl_3$) δ 215.86 d ($^1J_{CP}$ = 137.5), 44.60 t, 31.50 td ($^2J_{CP}$ = 52.0), 26.09 td ($^3J_{CP}$ = 4.3), 24.59 t.

[1-(Dipiperidin-1-yl-phosphinyl)-1-hydroxyethyl]-1-phosphonic acid dimethyl ester (1). Dimethyl phosphite (290 mg, 2.64 mmol) and dibutylamine (38.4 mg, 0.30 mmol) in ether (3 ml) were cooled to 0 °C. Compound **9** (568 mg, 2.20 mmol) in ether (2 ml) was slowly introduced at this temperature with stirring and the mixture was allowed to warm to the room temperature and refluxed for 2 hours. The resulting precipitate was filtered, washed with ether and dried *in vacuo* to give **1** (445 mg, 55%) as a slightly beige powder. ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, d, $^3J_{HP}$ = 10.4), 3.85 (3H, d, $^3J_{HP}$ = 10.4) 3.47 (–O*H*, t, $^3J_{HP}$ = 7.5), 3.28 (2H, m), 3.21 (4H, m), 3.10 (2H, m), 1.65 (3H, dd, $^3J_{HP}$ = 14.4, $^3J_{HP}$, = 16.5), 1.60 (4H, m), 1.55 (8H, m); ^{31}P NMR ($CDCl_3$) δ 29.17 d ($^2J_{PP}$ = 36.4), 24.36 d; ^{13}C NMR ($CDCl_3$) δ 74.51 dd ($^1J_{CP}$ = 116.6, $^1J_{CP}$, = 150.3), 54.24 qd ($^2J_{CP}$ = 7.2), 53.80 qd ($^2J_{CP}$ = 7.6), 45.93 t, 45.57 t, 26.17 td ($^3J_{CP}$ = 5.4), 26.03 td ($^3J_{CP}$ = 4.6) 24.76 t, 24.56 t, 20.70 q. Anal. calcd for $C_{14}H_{30}N_2O_5P_2$: C, 45.65; H, 8.21; N, 7.61%. Found: C, 45.77; H, 8.20; N, 7.64%.

[1-(Dipiperidin-1-yl-phosphinyl)-1-hydroxyethyl]-1-phosphonic acid piperidinyl methyl ester (2). Prepared similarly to **1** from **8** (500 mg, 3.06 mmol), **9** (712 mg, 2.76 mmol) and dibutylamine (60 mg, 0.46 mmol), but without cooling, only 5 ml of ether was used, and the reflux time was 40 h. The synthesis was carried out under a nitrogen atmosphere. Ether was removed *in vacuo.* The remaining mixture was dissolved in chloroform (2 ml) and hexane (10 ml) was added with rapid stirring. The solution was separated from a heavier syrup and evaporated in *vacuo.* The residue was purified by column chromatography using ethyl acetate–methanol (70 : 30) as an eluent (TLC R_f = 0.6, visualized by iodine) to give **2** (91 mg, 9%) as a slightly yellow syrup and still crude product (purity *ca.* 70%). Only one stereoisomer was observed. ^{1}H NMR ($CDCl_3$) δ 3.67 (3H, d, $^3J_{HP}$ = 11.1), 3.35–3.05 (12H, m), 1.62–1.48 (18H, m), 1.57 (3H, dd, $^3J_{HP}$ = 14.7, $^3J_{HP}$, = 16.9); ^{31}P NMR ($CDCl_3$) δ 30.52 d ($^2J_{PP}$ = 35.9), 27.81 d.

[1-(Dipiperidin-1-yl-phosphinyl)-1-hydroxyethyl]-1-phosphonic acid monomethyl ester monolithium salt (3). Compound **1** (191 mg, 0.52 mmol) and LiI (74 mg, 0.55 mmol) were dissolved in acetone (4 ml) and heated with stirring in an oil bath at 55 °C for 2.5 hours. The formed precipitate was filtered, washed with acetone and dried *in vacuo* to give **3** (145 mg, 78%) as a white powder. ^{1}H NMR (CD_3OD) δ 3.70 (3H, d, $^3J_{HP}$ = 9.9), 3.26 (6H, m) 3.17 (2H, m), 1.60 (3H, dd, $^3J_{HP}$ = 14.3, $^3J_{HP}$, = 15.6), 1.60 (4H, m) 1.54 (8H, m); ^{31}P NMR (CD_3OD) δ 34.13 d

($^2J_{PP}$ = 32.8), 18.81 d; ^{13}C NMR (CD_3OD) δ 76.49 dd ($^1J_{CP}$ = 120.2, $^1J_{CP}$, = 141.4), 53.71 qd ($^2J_{CP}$ = 6.8), 47.13 t, 47.06 t, 27.48 td ($^3J_{CP}$ = 5.3), 27.42 td ($^3J_{CP}$ = 5.3), 26.03 td ($^3J_{CP}$ = 4.5) 25.98 t, 25.84 t, 22.08 q. Anal. calcd for $C_{13}H_{27}LiN_2O_5P_2$: C, 43.34; H, 7.55; N, 7.78%. Found: C, 43.04; H, 7.48; N, 7.64%.

[1-(Dipiperidin-1-yl-phosphinyl)-1-hydroxyethyl]-1-phosphonic acid dipiperidinium salt (4). Compound **1** (100 mg, 0.27 mmol) was dissolved in trimethylsilyl bromide (1.0 ml) and stirred for 2 hours at room temperature. The mixture was evaporated *in vacuo* and the residue was dissolved in toluene with a few drops of piperidine added and then stirred 1 minute before evaporation to dryness. The residue was dissolved in dry acetone. Any solids were filtered and the filtrate was evaporated to dryness, then piperidine (1.5 ml) was added and the mixture was stirred for 30 minutes at room temperature. The piperidine was removed *in vacuo*, ether was added and the precipitate was filtered, washed with ether and dried in *vacuo* to give **4** (70 mg, 51%) as a slightly yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.23 (6H, m), 3.17 (2H, m) 3.00 (8H, t, J = 5.4), 1.71 (8H, m), 1.59 (3H, dd, $^3J_{HP}$ = 13.7, $^3J_{HP}$, = 15.5) 1.57 (8H, m), 1.51 (8H, m); ^{31}P NMR ($CDCl_3$) δ 34.00 d ($^2J_{PP}$ = 24.3), 18.42 d; ^{13}C NMR ($CDCl_3$) δ 74.31 dd ($^1J_{CP}$ = 121.3, $^1J_{CP}$, = 127.2), 46.02 t, 45.80 t, 45.04 t, 26.44 td ($^3J_{CP}$ = 5.0), 26.35 td ($^3J_{CP}$ = 4.5), 24.96 t, 24.86 t, 24.04 t, 23.48 t, 22.86 q.

[1-(Dipiperidin-1-yl-phosphinyl)-1-hydroxyethyl]-1-phosphonic acid monopiperidinyl ester monolithium salt (5). Prepared similarly to **3**, from **2** [(60 mg, 0.14 mmol (calculated as the pure compound)] and lithium iodide (20 mg, 0.15 mmol), but the reaction time was 25 h. **5** [(12 mg, 21% (calculated from pure **2**)] was obtained as a beige solid. ^{1}H NMR (CD_3OD): δ_H 3.29 (6H, m), 3.20 (6H, m) 1.60 (6H, m), 1.53 (12H, m), 1.49 (3H, dd, $^3J_{HP}$ = 14.6, $^3J_{HP}$, = 15.9); ^{31}P NMR (CD_3OD) δ 37.15 d ($^2J_{PP}$ = 32.9), 20.32 d;^{13}C NMR (CD_3OD) δ 78.17 dd ($^1J_{CP}$ = 111.2, $^1J_{CP}$, = 124.9), 47.09 t, 46.86 t, 30.51 t, 27.48 td ($^3J_{CP}$ = 5.1), 27.40 td ($^3J_{CP}$ = 4.1), 26.33 t, 26.04 t, 25.84 t, 23.48 t, 20.70 q.

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